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Supplemental information

Multimodal, broadly neutralizing antibodies against SARS-CoV-2 identified by high-throughput native pairing of BCRs from bulk B cells

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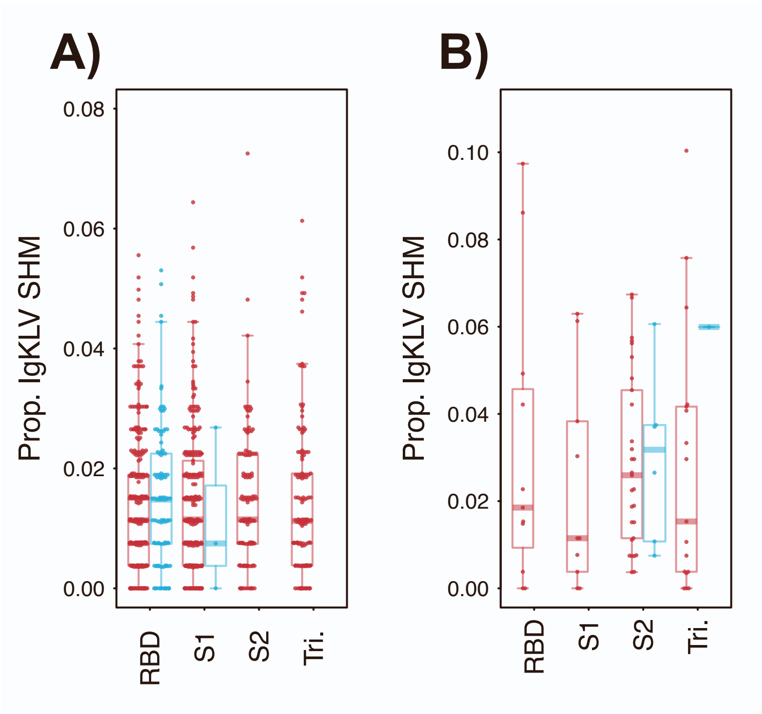
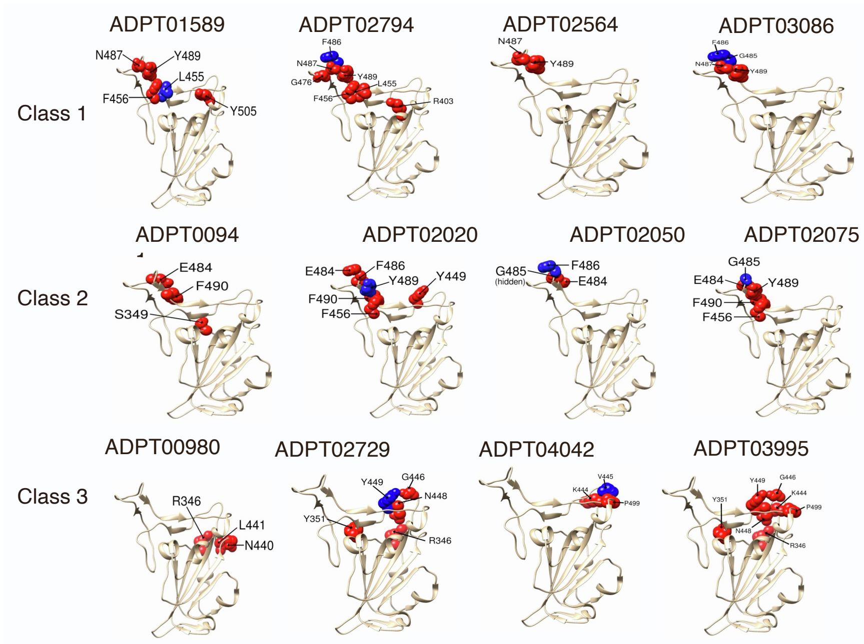


Figure S1: **Light chain SHM**. Related to Figure 1 and Figure 3.
Proportion of sites in light chain V genes in spike-reactive antibodies derived from antigen-enriched memory cells (A) and ASCs (B) that have experienced somatic hypermutation relative to germline.



NAb clone	Class	RBD Epitope Residues
ADPT01589	1	L455, F456, N487, Y489, Y505
ADPT02794	1	R403, L455, F456, G476, F486, F487, Y489
ADPT02564	1	N487, Y489
ADPT03086	1	G485, F486, N487, Y489
ADPT03091	1	F486, N487, Y489, P499
ADPT02892	1	Y351, R403, N448, L455, F456, Y489, Q493
ADPT02050	2	E484, G485, F486
ADPT02020	2	Y449, F456, E484, F486, Y489, F490
ADPT02075	2	E456, E484, G485, F490, Y490
ADPT00941	2	S349, E484, F490
ADPT00980	3	R346, N440, L441
ADPT02729	3	R346, Y351, K444, G446, N448, Y449, P499
ADPT04042	3	K444, V445, P499
ADPT03995	3	R346, Y351, K444, G446, N448, Y449, P499
ADPT04441	3	K444, V445, P449, N448, P499

● = **Critical** binding residue (reduces binding to <25% of WT)

● = Secondary binding residue

Figure S2: **Identification of critical clones for antibody binding to RBD.** Related to Figure 2.
Binding of each test antibody to each mutant clone in the alanine scanning library was measured in duplicate by high-throughput flow cytometry. To identify critical residues (red circles), a threshold of >70% WT binding to control antibody and <20% WT binding to test antibodies was applied. Secondary residues (blue circles) are highlighted for clones that did not meet the set thresholds but whose decreased binding activity and proximity to critical residues suggested that the mutated residue may be part of the epitope. Summary list of both primary and secondary residues is provided in the table. NAb = Neutralizing Antibody; RBD = Receptor Binding Domain.

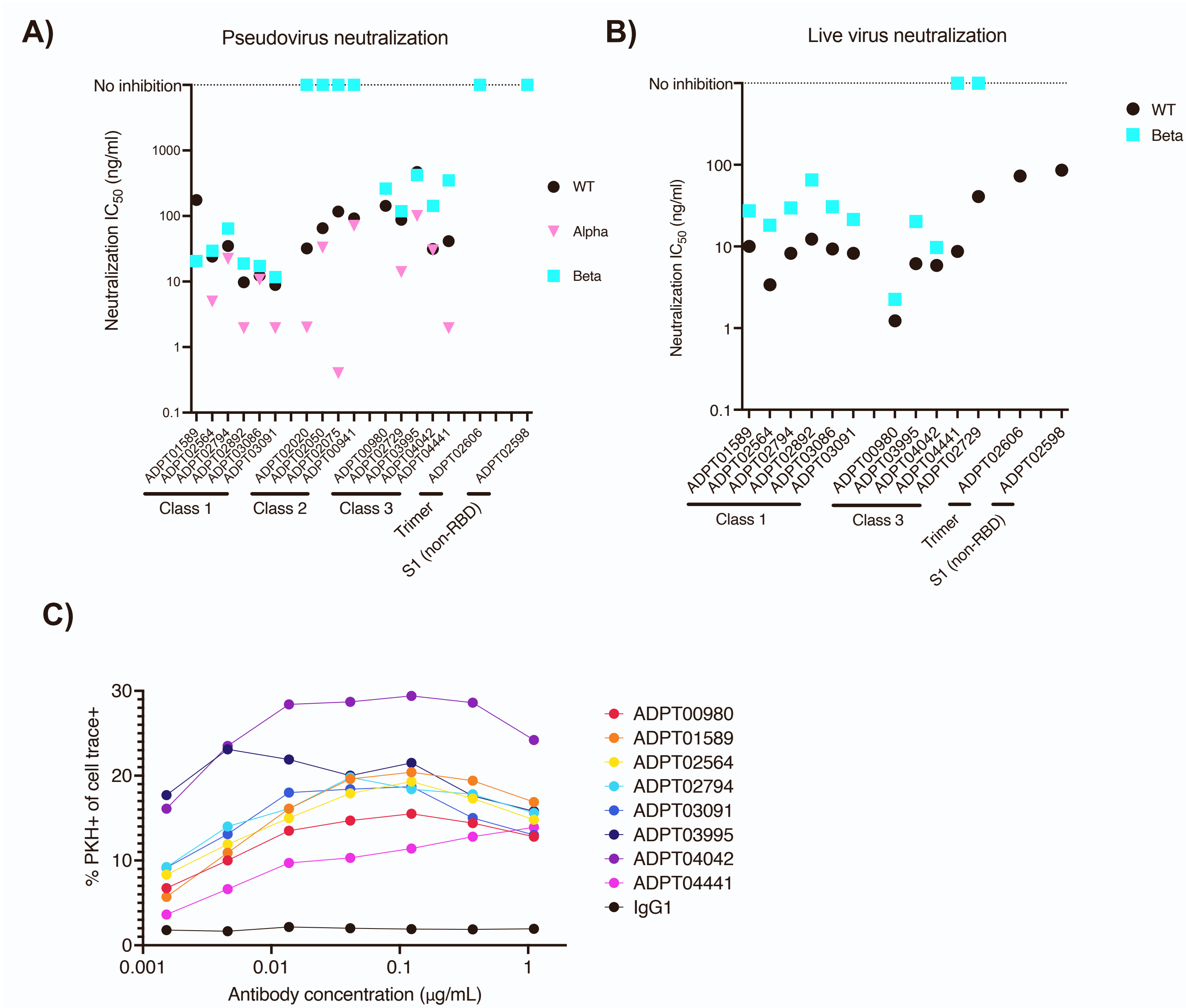


Figure S3: **Pseudovirus and Live virus neutralization and cell-based ADCP function.** Related to Figure 2. (A) Pseudovirus neutralization shown by IC50 of WT, Alpha, and Beta variants with lower assay sensitivity of 1,000 ng/mL (B) Live virus neutralization of WT and Beta variants, graph showing calculated IC50 values with a lower assay sensitivity of 100 ng/mL (C) Plot depicts ADCP activity of each antibody measured as the percentage of PKH67 fluorescent dye-positive events among the Cell Trace Violet dye-positive monocyte effector cells, assessed by flow cytometry. Monocytes were incubated with Cell Trace Violet dye and various amounts of antibody (X axis) and incubated with PKH67-labeled target cells (SARS-CoV-2 S glycoprotein stable HEK293T cells) prior to analysis.

ADCP = antibody-dependent cellular phagocytosis; PKH+ = PKH67 ethanolic dye solution positive

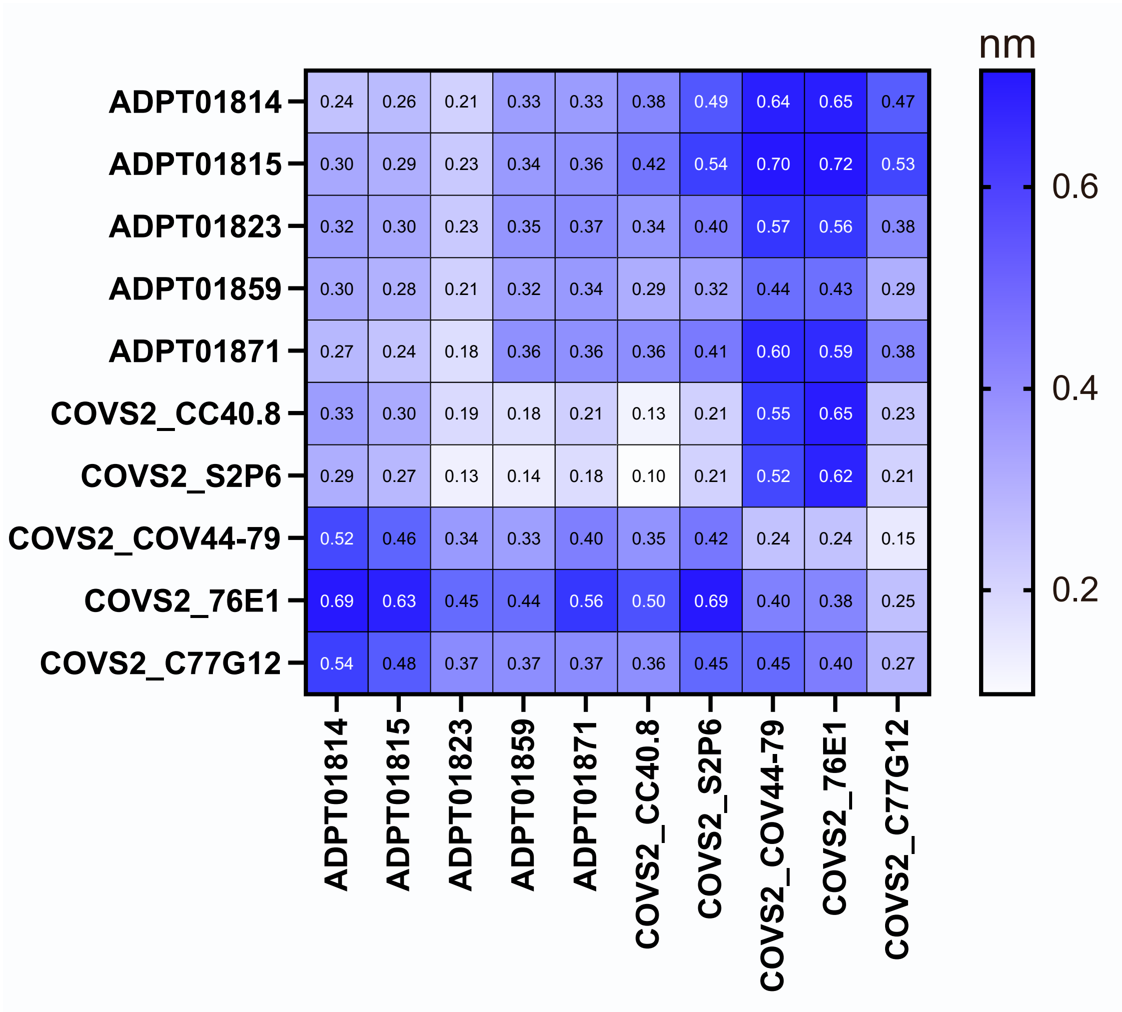


Figure S4: Epitope binning of S2 antibodies. Related to Figure 4.
Heatmap showing degree of binding interference between paired antibodies as determined by Octet competition experiments. CC40.8 and S2P6 are previously identified antibodies that bind to the stem helix region of S2 (Zhou, Yuan, et al. 2022; Song et al. 2021; Pinto et al. 2021) and COV44-79, 76E1, and C77G12 target the fusion peptide (Dacon et al. 2022; Sun et al. 2022; Low et al. 2022).

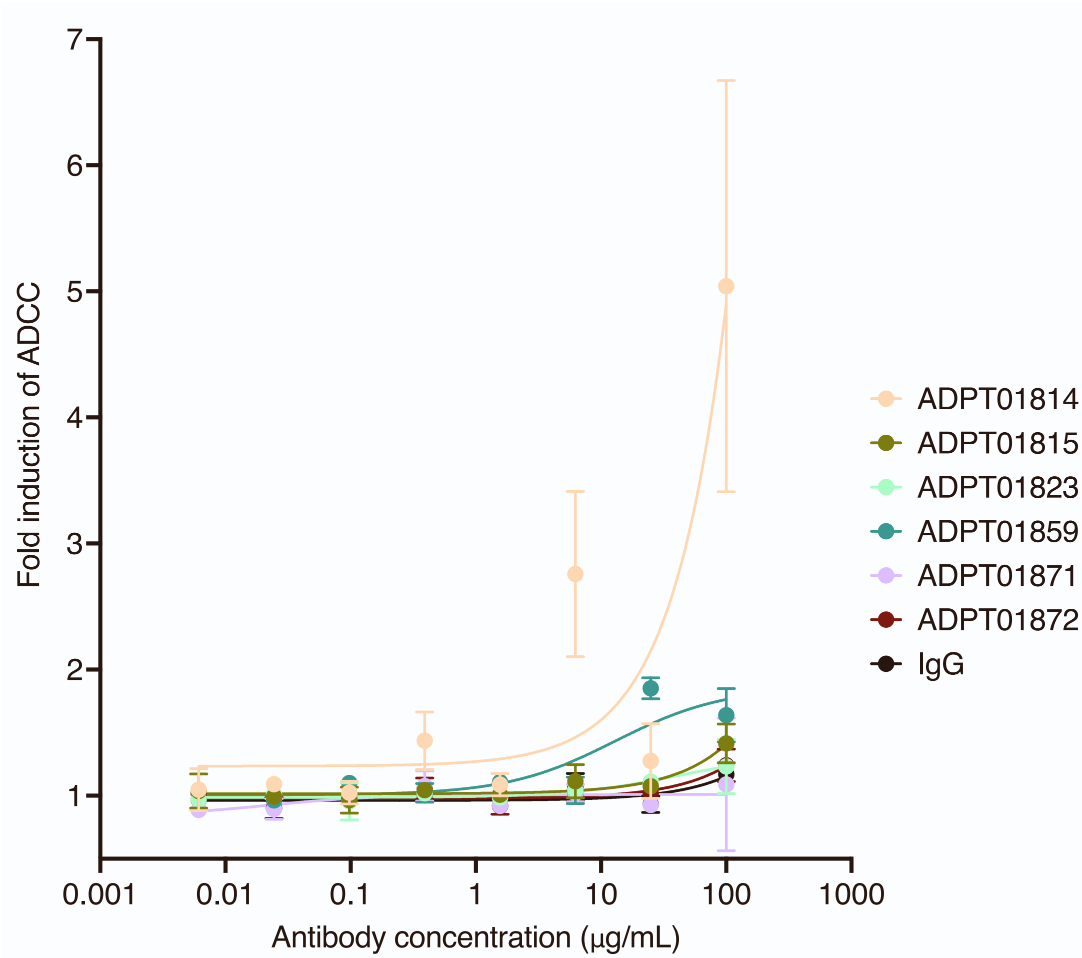


Figure S5: **Cell-based ADCC function**. Related to Figure 4.
In the ADCC assay, fold ADCC induction above background is shown as a measure of luciferase activity in the effector cells quantified with luminescence readout, at different antibody concentrations (X axis). ADCC = antibody-dependent cellular cytotoxicity.

Antibody Clone	COVID19 Specificity	Biacore K_d (pM)	Antigen binding EC_{50} (pM)	Potency live virus microneutralization IC_{50} (pM)
ADPT02793	S1	398	S1: 113 Trimer: 68	11.6
ADPT02025	S1	7,130	S1: 113 Trimer: 68	185
ADPT00937	S1	3,190	S1: 64 Trimer: 74	128
ADPT01238	S1	27	S1: 28 Trimer: 69	471
ADPT02854	Trimer	112	S1: no binding Trimer: 152	72
ADPT02019	Trimer	57	S1: no binding Trimer: 24	33
ADPT02024	Trimer	11700	S1: no binding Trimer: 665	95.1
ADPT02866	Trimer	154	S1: no binding Trimer:182	11
ADPT02597	S1	248	S1: 103 Trimer: 36	46
ADPT02928	S1	1420	S1: 58 Trimer: 24	52
ADPT03011	S1	4230	S1: 157 Trimer: 33	24
ADPT02646	S1	2.96	S1: 33 Trimer: 24	9
ADPT02381	Trimer	1020	S1: no binding Trimer: 33	18
ADPT02432	Trimer	243	S1: no binding Trimer: 24	11

Table S1: **Summary data of all the non-RBD antibodies characterized**. Related to Figure 2.
IC_{50} values for live virus neutralization represent an average of 3-4 experiments.

Locus	Name	Sequence
V	IGL_VL10-54-2_D5	CTGGGCTCTGCTCCTCCTGACCC
V	IGL_VL10-54-3_D7	CCTGGGTCATGCTCCTCCTGAAATCCTCAC
V	IGL_VL10-67_D6	GCTCCTCTCCTCCATCTCCACCCTCAC
V	IGL_VL11_D5	ATGGCCCTGACTCCTCTCCTCCTCC
V	IGL_VL1-40_D5	CTCCTCACTCTCCTCGCTCACTGCACAG
V	IGL_VL1-41_D5	TCTCCTCCTCACCCTTCTCATTCACTGCACAG
V	IGL_VL1-47_D5	CCTCTCCTCCTCACCCTCCTCACTCACTG
V	IGL_VL2-14_D5	TCTGCTGCTCCTCACTCTCCTCACTCGG
V	IGL_VL2-33_D5	CTCTGCTGCTCCTCACYCTCCTCACTCAG
V	IGL_VL2-34_D5	CTCTGCTCCTYCTCACCCTCCTCACTCAG
V	IGL_VL3-1_D5	GCATGGATCCCTCTCTTCCTCGGCGTC
V	IGL_VL3-10_D5	ATGGCCTGGAYCCCTYTCCTGCTC
V	IGL_VL3-12_D5	CCTCTCCTCCTCAGCCTCCTCGC
V	IGL_VL3-19_D5	GCCTGGACCCCTCTCTGGCTCAC
V	IGL_VL3-2_D5	CTCCCCTGCTCACCCTCCCGAC
V	IGL_VL3-21_D5	CTGGACCGTTCTCCTCCTCGGCC
V	IGL_VL3-22_D8	CACACTCCTGCTCCCACTCCTCAACC
V	IGL_VL3-24_D7	GGCCCCTCTTTTCCTTGGTGTCCTGAC
V	IGL_VL3-25_D5	CTCTACTTCTCCCCCTCYTCACTCTCTGCACAG
V	IGL_VL3-30_D5	ATGACCTTGCTGCAGGGTGAGGGG
V	IGL_VL3-9_D5	GCTCTCCTTCTGAGCCTCCTTGCTCACTTTACAG
V	IGL_VL4-3_D7	CCTGGGTCTCCTTCTACCTACTGCCCTTC
V	IGL_VL4-60_D5	CTCTTCCCTCTCCTCCTCCACTGCACAG
V	IGL_VL4-69_D5	CCTCACCCTCCTCCTCCACTGCACAG
V	IGL_VL5_D5	CTCTCCTCCTCCTGYTCCTCTCTCACTGC
V	IGL_VL6_D5	CACTACTTCTCACCCTCCTCGCTCACTGC
V	IGL_VL7_D5	CTCTCTTTCTGTTCCTCCTCACTTGCTGCCCAG
V	IGL_VL8_D5	GATGCTTCTCCTCGGACTCCTTGCTTATGGATCAG
V	IGL_VL9_D5	CCTGGGCTCCTCTGCTCCTCACC
V	IGL_VL5-52_D2	GGCCTGGACTCTTCTCCTTCTCGTGC
V	IGL_VL5-37_D3	TGGCCTGGACTCCTCTTCTTCTCTTGCTC
V	IGL_VL3-24_D9	CCTCTTTTCCTTGGTCTCCTGACTCACTGCC
V	IGK_VK1-12_D5	ATGGACATGATGGTCCCCGCTCAGCTC
V	IGK_VK1-16_D5	ATGGACATGAGRGTCCTCGCTCAGCTC
V	IGK_VK1-2_D5	TGAGGSTCCCYGCTCAGCTCC
V	IGK_VK1-22_D5	ATGGACATGAGGGTCCCCACTCAGCTC
V	IGK_VK1-27_D5	ATGGACATGAGGGCCCCCGCTC
V	IGK_VK1-43_D6	CTGCTGCTCTGGTTCCCAGGTGC
V	IGK_VK2-24_D5	TGCTCAGCTYCTGGGGCTGCTAATGC
V	IGK_VK3-11_D5	GCKCAGCTTCTCTTCCTCCTGCTACTCTG
V	IGK_VK3-25_D6	YCATTTCTTTATGTTACTCTGGGTCCCAGATTTCACTG
V	IGK_VK3-31_D6	AGCTCAGCTGCTTTGATTCTTGTTACTCTGGCTC
V	IGK_VK3-34_D6	CTCAGCTCCTCTCCTTTCTGGTACTCTGGCTC

V	IGK_VK3-7_D5	CCCAGCACAGCTTCTTCTTCCTCCTGC
V	IGK_VK4_D5	ATGGTGTTGCAGACCCAGGTCTTCATTTCTCTG
V	IGK_VK5_D7	CCTCAGCTTCCTCCTCCTTTGGATCTCTGATACC
V	IGK_VK6_D7	GCTCTGGGTTCCAGCCTCCAGG
V	IGK_VK7_D7	CTCCTGCTCTGGGCTCCAAGCTG
V	IGH_V1-17-01_D1	CTGGGGGATCCTCTTCTTGGTGGCATC
V	IGH_VH1L-17-02_D2	GGGGATCCTCTTCTTGGTGGCAGCTG
V	IGH_V3-22_D1	GGAGTCATGGCTGAGCTGGGTTTTTCTTGC
V	IGH_VH1L-03SS_D5	CCTCTTTTTGGTGGCAGCAGCCACAG
V	IGH_VH1L-18SS_D5	GCATCCTTTTCTTGGTGGCAGCAGCAAC
V	IGH_VH1L-45-2SS_D6	CTCTTCTTGGTGGCAGCAGCCACAG
V	IGH_VH1L-46-02SS_D1	GGTCTTCTGCTTGCTGGCTGTAGCACC
V	IGH_VH1L-46SS_D5	GGTCTTCTGCTTGCTGGCTGTAGCTCC
V	IGH_VH1L-58SS_D5	GTCCTCTTCTTGGTGGGAGCAGCGAC
V	IGH_VH1L-69SS_D5	CATGGACTGGACCTGGAGGTTCCTCTTTG
V	IGH_VH1L-8SS_D5	GGATCCTCTTCTTGGTGGCAGCAGCTAC
V	IGH_VH1L-FSS_D8	CCTCCTCTTGGTGGCAGCAGCTACAG
V	IGH_VH2L-05SS_D5	CACTTTGCTCCACGCTCCTGCTGC
V	IGH_VH2L-26SS_D5	GACACACTTTGCTACACACTCCTGCTGC
V	IGH_VH2L-5-4-6SS_D2	ATGGACATACTTTGTTCCACGCTCCTGCTGC
V	IGH_VH2L-70SS_D5	ATGGACATACTTTGTTCCACGCTCCTGCTAC
V	IGH_VH3L-09SS_D5	GTTGGGACTGAGCTGGATTTTCCTTTTGGC
V	IGH_VH3L-15-0102SS_D1	GGCTGAGCTGGATTTTCCTTSCTGC
V	IGH_VH3L-21SS_D5	GCTCCGCTGGGTTTTCCTTGTTGC
V	IGH_VH3L-23SS_D5	GGGCTGAGCTGGCTTTTTCTTGTGGC
V	IGH_VH3L-43SS_D5	GTTTGGACTGAGCTGGGTTTTCCTTGTTGC
V	IGH_VH3L-48SS_D1	GTTGGGGCTGTGCTGGGTTTTCCTTG
V	IGH_VH3L-64D-06SS_D1	GGAGTTCTGGCTGAGCTGGGTTCTCC
V	IGH_VH3L-72-49SS_D5	GAGTTTGGGCTKAGCTGGGTTTTCCTTG
V	IGH_VH3L-DSS_D5	TTGTGCTGAGCTGGGTTTTCCTTGTTGG
V	IGH_VH3L-FSS_D5	GGGCTGAGCTGGGTTTTCCTYGTTGC
V	IGH_VH4-34-05SS_D1	CTTCCTGCTCCTGGTGGCAGCTC
V	IGH_VH4-38-2-02SS_D1	GTTTTTCCTCCTGCTGGTGGCAGCTC
V	IGH_VH4-59-0102SS_D1	GGTTCTTCCTTCTCCTGGTGGCAGCTC
V	IGH_VH4L-1SS_D5	GTTCTTCCTCCTSCTGGTGGCAGCTC
V	IGH_VH4L-39SS_D1	GGTTCTTCCTCCTGCTGGTGGCG
V	IGH_VH4L-4-02SS_D1	GGTTCTTTCTCCTCCTGGTGGCAGCTC
V	IGH_VH5L-10-0-01-2SS_D1	GGGCCTCTCCACTTAAACCCAGGCTC
V	IGH_VH5L-51SS_D5	GGGTCAACCGCCATCCTCGC
V	IGH_VH6L-01SS_D5	CTCCTTCCTCATCTTCCTGCCCGTGC
V	IGH_VH6L-1-02SS_D3	TCTGTCTCCTTCCTCATCTTCCTGCCGTG
V	IGH_VH7L-04SS_D5	CACCATGGACTGGACCTGGAGGATCC
C	R_IGK_C	CATCAGATGGCGGGAAGATGAAGACAGATGGTGC
C	IGLC7-01_D03	CAGAGGAGGGCGGGAACAGAGTGAC

C	R_IGL_C	CCTCAGAGGAGGGTGGGAACAGAGTGAC
C	IGHA1_D03	GCTGGGTGCTGCAGAGGCTC
C	IGHA2_D06	AGCGGGAAGACCTTGGGGCTG
C	IGHD_D05	CCTGATATGATGGGGAACACATCCGGAGCC
C	IGHE_D08	CGGGTCAAGGGGAAGACGGATGG
C	IGHG1_D04	GTGCCAGGGGGAAGACCGATGG
C	IGHG4/G3/G2_D03	GCTCCTGGAGCAGGGCGC
C	IGHGP_D04	GCACCAGGGGGAAGACCGATGG
C	IGHM_D06	CGACGGGGAATTCTCACAGGAGACGAGG

Table S2: List of primers used for antibody heavy and light chain amplification. Related to Figure 1 s

Antibody Clone	Identifier	IgH	IgKL
ADPT00980	AB_2940970	QVQLVESGGGVVQPGRSLRLSCAASGFTFSRYGMHWVRQAPGKGLEWVALISSGGSNKYYADSVKGRFTISRDNSKNTLYLEMNSLRAEDTAVYYCAKDAIYDYIWGAYRENWFDPWGQGTLVTVSS	DIQMTQSPSSLSASVGDRVTITCRASQSISNYLNWYQQKPGKAPNLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQTSSPPLTFGQGTKVEIK
ADPT01589	AB_2940989	EVQLVESGGGLIQVGGSLRLSCAASGLTVTSNYMNWVRQGPGKGLEWVSLIYSGGTTYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCARPIVGARSGMDVWGQGTAVTVSS	DIQMTQSPSSLSASVGDRVTITCQASQDINKYLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLQPEDLATYYCHQFDNLPGTFGGGTKVEIK
ADPT01814	AB_2940990	QVQLQESGPGLVKPSETLSLTCSVSGGSINYYYWSWIRQTPGQGLEWIGFIYSSGTTNYNPSLKSRVTMSKDTAKKQFSLKLTSVTAADSAVYYCARHSRSCTNGVCQTYYYYALDVWGHGTTVTVSS	QSVLTQPPSVSGAPGQRVTISCTGSGSNIGSGYDVHWYQQLPGRAPKLLIYRNRNRPSGVPDRFSGSKSGTSASLAIAGLQSEDEGDYFCQSYDGRLGESAVFGGGTRLTVL
ADPT01815	AB_2940991	QVQLQESGPGLVKPSETLSLTCSVSGGSINYYYWSWIRKSPGKGLEWIGFIYSSGTTNYNPSLKSRVSMSIGTSKRQFSLKLSSVTAADSAVYYCARHSRSCTNGVCQTYYYYALDVWGHGTTVTVSS	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYANTHRPSGVPDRFSASKSGTSASLAIAGLQAEDEGDYYCQSYDGSLSESAVFGGGTRLTVL
ADPT01823	AB_2940992	EVQLVESGGGLVKPGGSLRLSCVASGFSFGLYTMNWVRQAPGKGLEWVSYISSSTSYKYYADSVKGRVSVSRDNAKNSLYLQLNGLRVEDTAVYYCARDGYCPNGICTYYGMDVWGQGTTVTVSA	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTITGLQSEDFAVYYCQQYDKWPPAYSFGQGTKVEIK
ADPT01859	AB_2940993	EVQLVESGGGLVKPGGSLRLSCAASGFSFNTYTMNWVRQAPGKGLEWVSYISSSSSYKYYSDSVKGRFSVSRDNAKKSLYLQMNGLRAEDTAVYYCARDGYCPNGVCTYYGMDVWGQGTTVTVSL	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISGLQSEDFAVYFCQQYSKWPPAYTFGQGTKLEIK
ADPT01871	AB_2940994	EVQLVESGGGLVKPGGSLRLSCVASGFSFSIYSMNWVRQAPGKGLEWVSYISSSSSYKYYADSVKGRFSVSRDNAKNSLYLQLNGLRAEDTAVYYCARDGYCPKGVCTYYGMDVWGQGTTVTVSA	EIVMTQSPATLSVSPGERVTLSCRASQSVRSRLAWFQQKPGQAPRLLIYDASIRATGIPARFSGSGSGTEFTLIISSLQSEDFAVYYCQQYDNWPPAYTFGQGTKLEIK
ADPT01872	AB_2940995	EVQLVESGGGLVKPGGSLRLSCAASGFSFSLYTMNWVRQAPGKGLEWVSYISSSSSYRYYADSVKGRFSVSRDNAKNALYLQMNGLRAEDTAVYYCARDGYCPRGVCTYYGMDVWGQGTTVTVSA	EIVMTQSPATLSVSPGERATLSCRASQSVGSRLAWYQQKPGQAPRLLIYDATIRATGIPARFSGSGSGTDFTLTISGLQSEDFAVYYCQRYNNWPPAYTFGQGTKLEIK
ADPT02564	AB_2940996	QVQLVQSGAEVKKPGSSVRVSCKASGGTFISYTFNWVRQAPGQGLEWMGRIIPIFGIVNYAQKFQGRVTIAADKSTSTAYMELSSLRSEDTAMYYCATATVDYDSGEEQSSFDPWGQGTLVTVSS	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKAGQTPRLLIYAASSRATGVPDRFSGSGSGTDFTLTISRLEAEDFAVYYCQQSWTFGQGTKVEIK
ADPT02598	AB_2940997	QVQLQESGPGLVKPSGTLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGETFHSGSFNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAIYYCATTRVGYEGHFYYYGMDVWGQGTTVTVSS	DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANRFPWTFGQGTKVEIK
ADPT02606	AB_2940998	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSITSSSGYMYYADSVKGRFTISRDNAKNSLYLQL	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQ

		NSLRAEDTAVYYCAKDSAFDLWEVRSYYYVMDVWGQGTTVTVSS	SEDFALYYCQQYNNWPRTFGQGTKLEIK
ADPT02794	AB_2940999	QVQLVQSGAEVKKPGSSLKVSCKASGGTFNNFAISWVRQAPGQGPEWMGRINPILSAAKYAQKFQGRLTITADKSTTTAYMELSSLRSEDTAVYYCAPTGTGESWWFDPWGQGTLVTVSS	QSVLTQPPSASGTPGQRVTISCSGSSSNIGTNYVYWYQQLPGTAPKVLIYGNNQRPSGVPDRFSGSKSGSSASLAISGLRSEDEADYYCAAWDDSLSGPVFGGGTKLTVL
ADPT03091	AB_2941000	QMQLVQSGPEVKKPGTSVKVSCKASGFTFSSSAVQWVRQARGQGLEWIGWIVVGSGNANYAQKLQERVSITRDMSTSTAYMELSSLRPEDTAVYYCAAPHCSRTICHDGFDMWGQGTMVTVSS	EIVLTQSPGTLSLSPGERATLSCRASQSVRSSYLAWYQQKPGQAPRLLMFVASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYDTSPWTFGQGTKVEIK
ADPT03995	AB_2941001	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSMHTIRWVRQAPGQGLEWMGRIIPMLGIVNYAQKFQGRVTISADKSTSTAYMELSSLTSEDTAMYYCAKGSHDVFDIWGQGTMVTVSS	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYNSYSPITFGQGTRLEIK
ADPT04042	AB_2941002	QITLKESGPTLVKPTQTLTLTCTFSGFSLSSGGVGVGWIRQPPGKALEWLALIYWDDDKRYRPSLKSRLTITRDTSTNQVVLTMTNMDPVDTATYFCARHQIATVFDHWGQGTLVTVSS	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYEVSNRPSGVSSRFSGSKSGNTASLTISGLQAEDEADYYCSSYTRSSPLVAFGGGTKVTVL
ADPT04441	AB_2941003	EVQLVESGGGLVQPGRSLRLSCAASGLTFEDYAMHWVRQPPGKGLEWVSGVSWNSGTIGYADSVKGRFTISRDNAKNSLYLHMRSLGAEDTAMYYCAKDMGGRFSFFSLENDAFDIWGQGTMVIVSS	SYELTQPPSVSVSPGQTARITCSGDALPKQSTYWYQQKPGQAPVLVIYKDIERPSGIPERFSGSSSGTTVTLTISGVQAEDEADYYCQSADSSDTYVFGTGTKVTVL

Table S3: List of antibody heavy and light chain sequences. Related to Figures 5 and 6

Supplemental References

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